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# Towards peptide-based inhibitors as therapies for Parkinson's disease

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The evidence for alpha-synuclein ( $\alpha$ -syn) as a key player in Parkinson's disease (PD) pathology is compelling despite the fact that the native function of the protein has yet to be fully elucidated[1]. For example, synthetic  $\alpha$ -syn aggregates that are characteristic of synucleinopathies lead to  $\beta$ -sheet rich amyloid structures similar to those found in Lewy bodies [2]. These structures lead to cell death with the majority of point-mutations associated with early onset PD (A30P, E46K, H50Q, G51D, A53T) clustered within a small region of the SNCA gene, which influence the rate and extent of aggregation which correlates with toxicity [3-6]. Until recently it was thought that the native state of  $\alpha$ -syn was structurally disordered [7] before undergoing a structural conversion to  $\beta$ -sheet and amyloid. However, recent albeit controversial findings suggest that the native state is a monomer which self-associates on lipids to form a helical tetramer, that exists in the crowded molecular environment of the cell but does not accumulate *in vitro* [8]. Several  $\alpha$ -syn point mutations have been hypothesized to destabilise this proposed tetramer, leading to increased levels of monomer which then aggregate [9]. As with the A $\beta$  peptide associated with Alzheimer's disease [10], recent studies have found that the heterogeneity of symptoms in synucleinopathies varies according to structurally different  $\alpha$ -syn aggregates. Differences in conformation and oligomeric state have been associated with different neurotoxic species and may define different phenotypes [11].

Inhibitor design is most effective on the basis of detailed knowledge of the structure and function of a target protein. In the absence of a well-defined native structure for  $\alpha$ -syn, many researchers have turned their focus toward the rational design of  $\beta$ -sheet breakers that can bind to and sequester the more accepted  $\beta$ -sheet precursors to amyloid formation. Amyloid structures are known to consist of multiple beta-strands that are stabilised by intermolecular interactions. These protein-protein interactions usually involve shallow surfaces stabilised by many points of contact and tend to lack the well-defined hydrophobic ligand-binding pocket most amenable to small drug-like inhibitors that might prevent or reverse amyloid formation [12]. Coupled with the resurgence of interest in peptide-based drug discovery, many groups have therefore turned to peptides to target such protein-protein interactions [13]. Short peptides are composed of natural amino acids and so their degradation is less likely than synthetic small organic molecules to be toxic, and less likely than proteins to be immunogenic since they fall below the threshold. In addition, they can now be quickly and cheaply synthesised

by chemical means and can undergo increasingly well-understood modifications that maintain desired binding properties but confer advantages such as improved membrane permeability, greater protease resistance and reduced plasma clearance rates. In relation to  $\alpha$ -syn aggregation, peptide-based inhibitors have included  $\beta$ -strand structures that bind  $\alpha$ -syn on one face and prevent  $\beta$ -sheet extension by virtue of N-methyl groups or other moieties on another face [14]. This strategy brings resistance to the action of proteases while increasing  $\beta$ -sheet propensity, thereby pre-organizing the molecule into an optimal  $\alpha$ -syn binding structure. Other inhibitors have included short peptides based on the 69-72 region of  $\alpha$ -syn that have included a polyarginine appendage to assist cell uptake [15]. Another approach has used a fragment of the  $\beta$ -syn peptide to prevent  $\alpha$ -syn oligomerisation [16]. In all these examples, the focus has been confined to studying  $\alpha$ -syn residues 71-82. This hydrophobic self-recognition element has been found to be toxic in isolation and is considered the key template for amyloid formation in the full-length  $\alpha$ -syn protein [17, 18].

Perhaps a more logical approach would be to stabilise either a defined monomeric structure or, if it exists, the helical tetrameric conformation, so as to prevent  $\alpha$ -syn misfolding in the first instance by functioning as a kinetic stabiliser of a non- $\beta$ -sheet and non-amyloid conformation. This may be a better strategy than searching for oligomer inhibitors, as these species can be difficult to define and therefore specifically target. This method has been used successfully via small molecules to stabilise the native dimeric form of transthyretin amyloidosis (ATTR) to prevent neuropathy and/or cardiomyopathy [19]. Model peptides have previously been locked into  $\alpha$ -helical tetramers on a scaffold, thereby regulating the rate of helix-sheet-amyloid conformational transitions and there is evidence of such templated helix bundles inhibiting  $\beta$ -sheet aggregation and amyloid formation [20, 21]. Other recent work utilises intracellular peptide library screening using a split reporter protein to identify inhibitors of  $\alpha$ -syn aggregation [22]. This approach has been taken to circumvent the absence of a well-defined target structure, or indeed oligomeric state. Therefore no assumptions need be made regarding the conformation of the protein, the mode of binding, or the oligomeric state populated as a consequence. Rather, by screening libraries inside the cell, peptides derived using this approach were only selected if they bound to  $\alpha$ -syn and conferred cell survival by lowering associated toxicity. This last point is crucial and has hindered the search for effective  $\beta$ -sheet breakers. Intracellular screening ensures that both target and library are expressed under native folding conditions within the crowded environment of the cell. Therefore peptides that are themselves toxic, bind to other proteins, form amyloid or are susceptible to proteases will tend to be rapidly cleared using this *in vivo* approach, and will complicate *in vitro* assays that need to be multiplexed screening systems. In our experiments, the libraries have usually been based on the 46-54 region of  $\alpha$ -syn where the majority of mutations associated with early onset PD are found. In these experiments the identified inhibitors have been found to prevent  $\alpha$ -syn amyloid formation and lead to significant reductions in toxicity.

There are some exciting advances in the development of peptides and mimetics to bind to and prevent toxicity associated with  $\alpha$ -syn amyloid formation. Coupled with recent developments in structural information relating to amyloid-based systems [23], and the increasing ability to readily modify peptides to deal with limitations in their druggability, there is now considerable optimism for peptide-based drug discovery for  $\alpha$ -syn.

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